

Sig2Lead Ver. 1.0

User Manual

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Sig2Lead Overview

Sig2Lead aims to facilitate drug discovery and re-purposing by connecting small molecule and target gene knock-down (KD) transcriptional signatures generated by the LINCS consortium. For a target gene specified by the user, putative inhibitors are identified as those drug-like molecules in LINCS that have signatures concordant with a KD signature of the target. Note that LINCS arguably represents the largest resource for pharmacogenomics to date, with over 20,000 small molecules and about 5,000 gene KDs transcriptionally profiled, thus covering a large subset of the drug-like chemical space and druggable subset of the genome. Furthermore, if a set of candidate molecules is provided, e.g., identified by virtual or experimental screening, these external candidate molecules are ranked based on their chemical similarity to 'concordant' LINCS analogs using a fast chemical similarity search. Additionally, Sig2Lead can be used to prepare input files for docking simulations to be performed in conjunction with connectivity-based analysis to improve the specificity of the search (see paper below), as well as identify LINCS analogs irrespective of their connectivity to a target of interest.

Reference

The Sig2Lead application implements the signature connectivity analysis for drug discovery found in the following paper:

[Thorman, A. W., Reigle, J., Chutipongtanate, S., Shamsaei, B., Pilarczyk, M., Fazel-Najafabadi, M., ... & Meller, J. \(2020\). Accelerating Drug Discovery and Repurposing by Combining Transcriptional Signature Connectivity with Docking. *bioRxiv*.](#)

Paper Abstract

The development of targeted treatment options for precision medicine is hampered by a slow and costly process of drug screening. While small molecule docking simulations are often applied in conjunction with cheminformatic methods to reduce the number of candidate molecules to be tested experimentally, the current approaches suffer from high false positive rates and are computationally expensive. Here, we present a novel *in silico* approach for drug discovery and repurposing, dubbed *connectivity enhanced* Structure Activity Relationship (*ceSAR*) that improves on current methods by combining docking and virtual screening approaches with pharmacogenomics and transcriptional signature connectivity analysis. *ceSAR* builds on the landmark LINCS library of transcriptional signatures of over 20,000 drug-like molecules and ~5,000 gene knock-downs (KDs) to connect small molecules and their potential targets. For a set of candidate molecules and specific target gene, candidate molecules are first ranked by chemical similarity to their 'concordant' LINCS analogs that share signature similarity with a knock-down of the target gene. An efficient method for chemical similarity search, optimized for sparse binary fingerprints of chemical moieties, is used to enable fast searches for large libraries of small molecules. A small subset of candidate compounds identified in the first step is then re-scored by combining signature connectivity with docking simulations. On a set of 20 DUD-E benchmark targets with LINCS KDs, the consensus approach reduces significantly false positive rates, improving the median precision 3-fold over docking methods at the extreme library reduction. We conclude that signature connectivity and docking provide

complementary signals, offering an avenue to improve the accuracy of virtual screening while reducing run times by multiple orders of magnitude.

Installation of Sig2Lead_v1

Introduction

Sig2Lead is available as a Shiny app to be executed in RStudio or as a docker container to be executed in a system-independent manner. The RStudio version is available on Github and the dockerized version is available on Docker Hub. The RStudio version requires that R and RStudio be locally installed on the user's computer and requires the installation of all requisite R packages. The dockerized version requires installation of docker and download of Sig2Lead image. Once the Sig2Lead image is running, a web browser is used to run the app without the need to install R, RStudio, or all necessary R packages. This manual provides instructions for both versions.

Installation/Configuration of RStudio Version

A. Install R and RStudio

Sig2Lead was built with R version 4.0.3 and is expected to run with any later versions of R (to avoid R dependencies please see dockerized version). The latest version of R can be downloaded at:

<http://www.r-project.org/>

Additionally, RStudio is required and can be downloaded at:

<https://www.rstudio.com/products/rstudio/download/>

B. Download Sig2Lead from Github

Sig2Lead and associated files can be downloaded from:

https://github.com/sig2lead/sig2lead_v1/

Navigate to the Sig2Lead_v1 repository, click on "Code", and select "Download Zip". The downloaded zip file will then need to be unzipped to a user-selected directory.

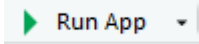
C. Install Required Libraries

Once R and RStudio are installed, shiny must be installed. This can be completed by typing into the R console:

```
install.packages("shiny")
```

All other dependencies and libraries will be installed upon the first time running the application.

This step may not be handled properly on MacOS, requiring a step-by-step installation of missing libraries. An alternative for Mac users is to use the dockerized version that takes care of all the dependencies.

To run the application, click the  button at the top middle of the RStudio interface.

Installation of Dockerized Version

A. Install Docker

Ubuntu: follow [the instructions](#) to get Docker CE for Ubuntu.

Mac: follow [the instructions](#) to install [the Stable version of Docker CE](#) on Mac.

Windows: follow [the instructions](#) to install [Docker Toolbox](#) on Windows.

For list of useful docker commands please see:

<https://www.digitalocean.com/community/tutorials/how-to-remove-docker-images-containers-and-volumes>

B. Download Sig2Lead Container from Docker Hub

To download the docker image run the following command in a command prompt:

```
[sudo] docker pull reiglej/sig2lead:v1
```

Linux users may need to use sudo to run Docker.

Note that the Sig2Lead image is available from Docker Hub at

<http://hub.docker.com/repositories/Sig2Lead/>

C. Run Sig2Lead Container

To run Sig2Lead container, open a command prompt and run following command:

```
docker run -p 3838:3838 -v <local_path>:/srv/shiny-server/userfile -d reiglej/sig2lead:v1
```

The <local_path> is the path of the local directory that contains the smiles or sdf file of your added compounds.

For Windows, the following line is an example with local directory "C:\Added_Compounds"

```
docker run -p 3838:3838 -v C:\Added_Compounds:/srv/shiny-server/userfile -d  
reiglej/sig2lead:v1
```

For MAC/UNIX, the following line is an example with local directory "/User/Added_Compounds"

```
docker run -p 3838:3838 -v /User/Added_Compounds:/srv/shiny-server/userfile -d  
reiglej/sig2lead:v1
```

Before running the container make sure that port 3838 is free to run. You can stop and kill any other docker containers on this port by

```
[sudo] docker stop <container ID> && docker rm <container ID>
```

To check the container ID run this command:

```
docker ps -a
```

D. Open browser and navigate to localhost:3838

Open browser and navigate to the following url: <http://127.0.0.1:3838>

E. Run app following instructions below

Follow the instructions below for running the app.

Connectivity Analysis Tab

In the next several sections, several distinct workflows organized in 3 different tabs are discussed and illustrated using use cases. The first of those sections below describes the main tab where the primary workflow can be initiated by defining the target gene.


A. Define Target Gene Workflow

This is the standard workflow for identifying small molecule inhibitors or activators of a target of interest. Within this workflow, a gene of interest is required and optionally, a user-defined list of compounds for scoring can be provided in SDF or SMILES format. Sig2Lead collects all data from genetic knockdowns within LINCS and identifies compounds that generate highly concordant transcriptional signatures to these genetic knockdowns, i.e., putative target/pathway inhibitors. If an external set of candidate molecules is provided, e.g., identified by virtual or experimental screening, these user-provided candidate molecules are ranked based on their chemical similarity to 'concordant' LINCS analogs using a fast chemical similarity search. This method allows scoring of small molecules to be tested for the purpose of library reduction. Results of added compounds will be ranked in descending order of similarity to LINCS analogs, with ties broken by concordance scores in the table titled "My Candidates Ranked" and compounds identified from within the LINCS library will be scored only by their concordance values to a knockdown of the target gene of interest in the

table titled “LINCS Compounds Ranked”. These tables can be downloaded using the download buttons just below the “Go!” button.

Sig2Lead

Signature Connectivity Analysis Chemical Similarity Analysis Network Analysis Help



Define Target Gene

Input a Target Gene

bcl2a1

Add candidate compounds in SMILES or SDF (Optional)

Browse... A1Compounds.sml

Upload complete

Show Advanced Options

Go!

My Candidates Ranked

LINCS Candidates Ranked

Show: 10 entries

Search:

My Candidates Ranked

User-added Compound	LINCS Analog	Cell Line	Concordance	Similarity
ZINC000003953830	LSM-5529	HT29	0.245	1
ZINC000001717014	LSM-4706	HT29	0.315	0.948
ZINC000001703010	LSM-36810	MCF7	0.311	0.948
ZINC000005029790	LSM-37142	NPC	0.218	0.946
ZINC000001715674	LSM-4706	HT29	0.315	0.937
ZINC000001600320	LSM-1873	A375	0.251	0.933
ZINC000004769009	LSM-5309	A549	0.248	0.925
ZINC000005707314	LSM-2332	A375	0.204	0.924
ZINC000014614772	LSM-43087	HCC515	0.257	0.92
ZINC000001608855	LSM-5381	SKB	0.346	0.913

Showing 1 to 10 of 158 entries

Previous 1 2 3 4 5 ... 16 Next

Show: 10 entries

Search:

LINCS Candidates Ranked

Candidate Name	Candidate LSM ID	Cell Line	Concordance
Valyltryptophan	LSM-3021	VCAP	0.566
MEGXP0_001444	LSM-4390	VCAP	0.561

Figure 1: Define Target Gene workflow. In this primary workflow, the user defines a gene of interest to identify putative pathway/target inhibitors. If the user defines a set of compounds from some other analyses, those compounds will be compared to the LINCS library for analogous compounds and scored by similarity and concordance in the upper table (My Candidates Ranked), which will only appear in the event of added compounds. The lower table (LINCS Candidates Ranked) will always appear when running this workflow and scores all LINCS compounds with a concordance above the threshold in descending order of concordance.

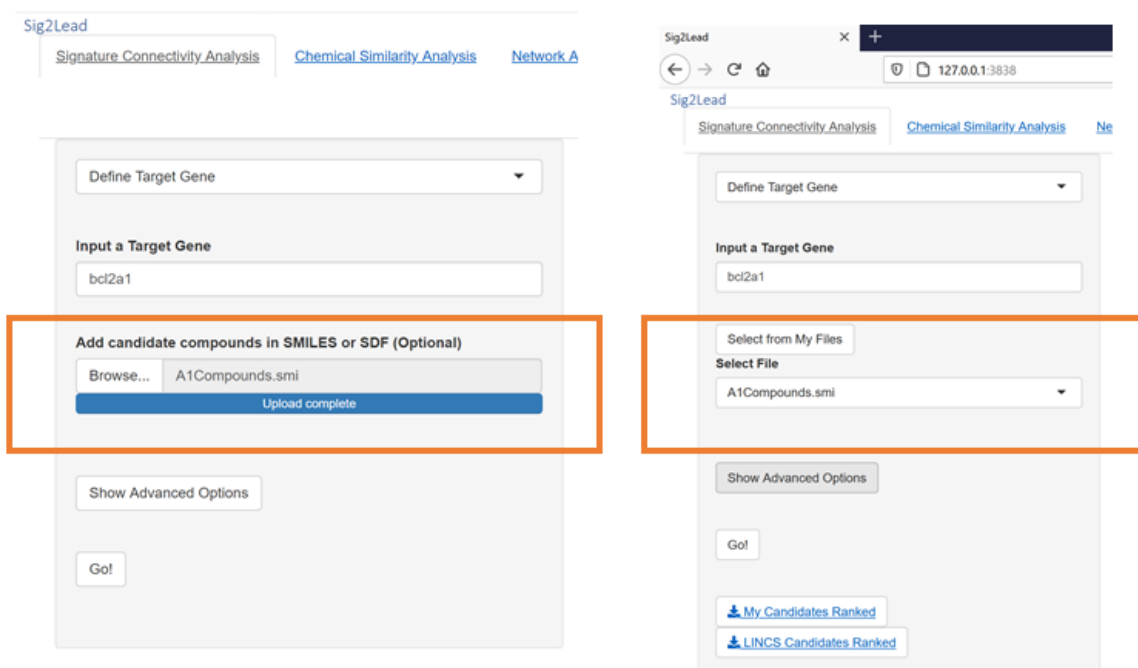


Figure 2: The two file input controls for the RStudio version (left) and the dockerized version (right) are shown in the above figure. The RStudio version (left) has a standard upload control in which the user can press the “Browse” button and navigate to the directory with the “.smi” or “.sdf” files of user-provided compounds. The file input control for the dockerized version (right) is slightly different with a button labeled “Select from My Files” which when pressed lists “.smi”, “.txt”, and “.sdf” files in the user’s local mounted directory (instructions for mounting a directory when launching the dockerized version are described in the ‘Installation of Dockerized Version’ section) which the user selects.

B. Upload a Signature Workflow

This workflow allows users to upload a signature of their own to search for potential inhibitors to a target gene unavailable on LINCS, or molecules that generate a similar signature to some other system perturbation that is otherwise undefined. In this workflow, users define a signature using one of the formats defined at (www.ilincs.org/ilincs/signatures/main/). This pipeline otherwise follows the same pipeline as the “Define Target Gene” workflow.

The screenshot shows the Sig2Lead web application. The sidebar on the left contains the following elements:

- Navigation tabs: Signature Connectivity Analysis, Chemical Similarity Analysis, Network Analysis, Help.
- Buttons: Upload a Signature, Select from My Files, Select File (with a dropdown menu showing 'sample2.txt'), Show Advanced Options, Go!
- Links: My Candidates Ranked, LINCS Candidates Ranked.

The main panel displays the results of the upload workflow:

Show 10 entries Search:

LINCS Candidates Ranked

Candidate Name	Candidate LSM ID	Cell Line	Concordance
CHEMBL179288	LSM-3648	A549	0.332
Hinokitiol	LSM-2835	MCF7	0.33
CHEMBL2142877	LSM-29356	MCF7	0.323
AC1MFIS3	LSM-6420	MCF7	0.314
CID 75060944	LSM-36674	HCC515	0.312
CID 6167421	LSM-44973	MCF7	0.31
BRD4770	LSM-42692	A375	0.305
ML228	LSM-2978	MCF7	0.303
Benzylpenicillin	LSM-3229	A375	0.301
AC1OAV9H	LSM-6653	A549	0.3

Showing 1 to 10 of 427 entries

Previous 1 2 3 4 5 ... 43 Next

Figure 3: Upload a Signature Workflow. This workflow allows one to query iLINCS (see ilincs.org) to find small molecules in LINCS that have signatures concordant with a user-provided transcriptional signature. The signature file should be saved as a tab-separated text file with gene symbol, log of differential expression value, and p-value comprising the columns in that order. The output of this workflow is a table of concordant LINCS small molecules to the uploaded signature.

C. Find Analogs in LINCS Workflow

This workflow simply identifies LINCS analogs to user-defined compounds, irrespective of their connectivity to a target. This requires added compounds and is useful in determining if there are transcriptionally profiled analogs in the LINCS library. LINCS compounds are all drug or drug-like, so this may be a simple filter to remove compounds that do not contain normal drug-like structures. Additionally, it was used in benchmarking as a baseline similarity of various compound libraries to the LINCS small molecules.

Sig2Lead

Signature Connectivity Analysis **Chemical Similarity Analysis** Network Analysis Help

Find Analogs in LINC5

Add candidate compounds in SMILES or SDF (Optional)

Browse... A1Compounds.sml

Upload complete

Show Advanced Options

Number of Analogs

3

Chemical Similarity Search

minSim

Chemical Similarity Threshold

0.65

Go!

Analogs in LINC5

Show: 10 entries

Search:

User-added Compound	LINC5 Analog	Similarity
ZINC000000056435_1	LSM-25663	1
ZINC000000056435_1	LSM-15355	0.938
ZINC000000056435_1	LSM-19955	0.8
ZINC000000056435_2	LSM-25663	1
ZINC000000056435_2	LSM-15355	0.938
ZINC000000056435_2	LSM-19955	0.8
ZINC0000000344612	LSM-24086	0.818
ZINC0000000344612	LSM-21266	0.795
ZINC0000000344612	LSM-26573	0.792
ZINC0000000551644	LSM-5331	0.744

Showing 1 to 10 of 447 entries

Previous 1 2 3 4 5 ... 45 Next

Figure 4: Find Analogs in LINC5 Workflow. This workflow allows the user to find small molecules included in LINC5 library that are structurally similar to user-provided compounds. The Advanced Options to change the number of LINC5 analogs to be returned for each user-provided compound bounded by the chemical similarity threshold (also adjustable). The user can also use either the ultrafast minSim algorithm or slower fpSim algorithm to compute chemical similarity.

D. Advanced Options

- Users can select to identify activators instead of inhibitors. Functionally, this is searching for molecules that are discordant to the gene knockdown of interest instead of concordant.
- Users can change the method of similarity search. The default, minSim is an exact fast chemical similarity search utilized by Sig2Lead. This is a novel contribution within Sig2Lead that is currently unpublished as its own method. For comparison, an established fpSim function to compute chemical similarity is available as a slow option (it typically runs about 100x slower than minSim).
- Finally, users can change the concordance threshold. The default of 0.2 is the minimum allowed threshold and corresponds to the threshold used in benchmarking for the ceSAR publication. For some targets, a more stringent cutoff may be required to increase the specificity. The range of these concordances can theoretically be from -1 to 1, but realistically below 0.2 have no significant concordance and very few generate a concordance above 0.5 or 0.6 (see the Sig2Lead/ceSAR manuscript).

Figure 5 shows a screenshot of the 'Show Advanced Options' panel in the Define Target Gene Workflow. The panel is highlighted with an orange border and contains three sections:

- Activation or Inhibition:** A dropdown menu set to 'Inhibit'.
- Chemical Similarity Search:** A dropdown menu set to 'minSim'.
- Concordance Threshold:** A text input field set to '0.2'.

Figure 5. Define Target Gene Workflow Advanced Options.

Chemical Similarity Analysis Tab

After searching LINCS for analogs, compounds can be further analyzed through chemical similarity using the “Chemical Similarity Analysis” tab. This would be done as a standard if only Sig2Lead is run for compound identification but is not necessary to run a ceSAR analysis as described in the linked publication. To run chemical similarity clustering click the “Run SAR” button. This will initiate a clustering analysis that compares concordant compounds from LINCS for the target and user-added compounds and clusters them by chemical similarity to one another. By default, 5,000 compounds will be analyzed (this can be changed under advanced options if an extended time is acceptable). The output will be in the form of two figures, a heatmap and an MDS plot, and a table of centroids for each cluster. The heatmap is generated through hierarchical clustering and shows a distance matrix comparing each compound identified through LINCS (Green) or added by the user (Blue). The MDS plot is an alternative view of the hierarchical clustering showing relative distances between clusters of compounds. The radius of each pie chart corresponds to the size of the cluster.

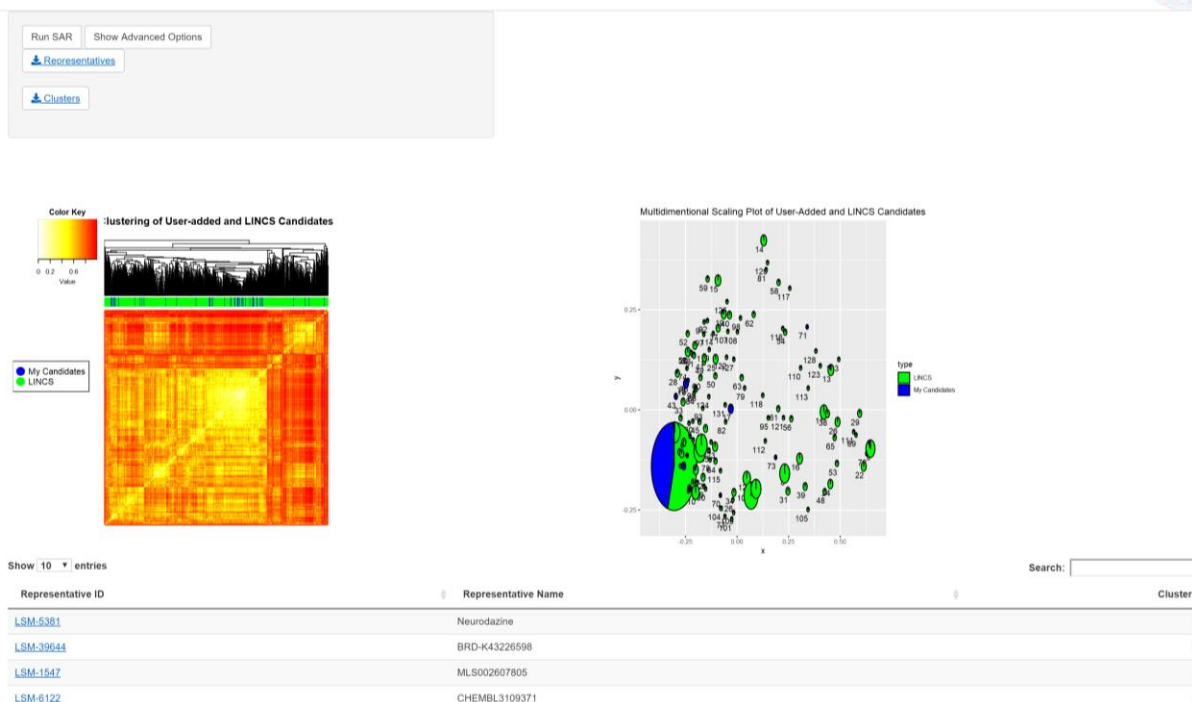


Figure 6. Example of chemical similarity analysis. SAR type analyses can be performed for either ‘concordant’ LINC compounds (here shown in Green), or user provided candidate molecules (here in Blue), or together to identify distinct classes of chemical moieties within the set of user provided compounds and their ‘concordant’ LINC analogs. The heatmap in the left panel shows pairwise chemical similarity pattern with the compounds analyzed as both columns and rows, i.e., the diagonal represents identity (Tanimoto coefficient of 1.0). Note that user defined candidates indicated by blue ticks in the top bar are scattered throughout, i.e., they belong to several distinct classes of compounds with most of them in the middle ‘big’ cluster indicated by a large rectangular block of high similarity scores in the middle of the heatmap. This is further highlighted in the right panel that shows individual clusters identified using the MDS 2-dimensional projection of pairwise similarities, with the ‘big’ circle in the bottom left corner corresponding to the central mixed cluster in the heatmap.

A. Chemical Similarity Analysis Options

- For clustering, different Tanimoto similarity thresholds can be selected. The default is 0.75, which groups compounds of modest difference together, but can be adjusted to make more (closer to 1) or less stringent clusters (closer to 0). This will only be reflected in the MDS plot and the table of representatives.
- The minimum cluster size for inclusion in the MDS plot can also be changed. By default, compounds that are not structurally related to at least two others at the specified threshold are independent and a representative of that group cannot be retrieved. The user can instead

cluster all compounds regardless of cluster size or increase this threshold to only consider those from large clusters of related compounds.

The screenshot shows the 'Chemical Similarity Analysis' tab in the Sig2Lead application. At the top, there are navigation links: 'Signature Connectivity Analysis', 'Chemical Similarity Analysis' (which is highlighted), 'Network Analysis', and 'Help'. Below these links, there are two buttons: 'Run SAR' and 'Show Advanced Options'. The 'Show Advanced Options' button is highlighted with an orange border. Below the buttons, there is a section titled 'Advanced Options' which is also highlighted with an orange border. This section contains three input fields: 'Tanimoto Similarity' with a value of 0.75, 'Minimum Cluster Size' with a value of 3, and a checkbox labeled 'Cluster all Compounds' which is currently unchecked.

Figure 7. Chemical Similarity Analysis Advanced Options.

Network Analysis Tab

Finally, Network connectivity analyses can be performed after chemical similarity analyses using STITCH on the “Network Analysis” tab. This analysis is intended to scrape any known information about identified compounds and their interactions with members of the pathway of interest. This step can be performed either through a global view (all identified compounds) or on a cluster by cluster basis (much faster). When running Global STITCH analysis, only the compounds found in clusters of sufficient size, as determined on the “Chemical Similarity Analysis” tab’s advanced options will be included unless the “Shows all clusters” box is checked. The Global STITCH analysis can be slow, depending on the number of compounds added.

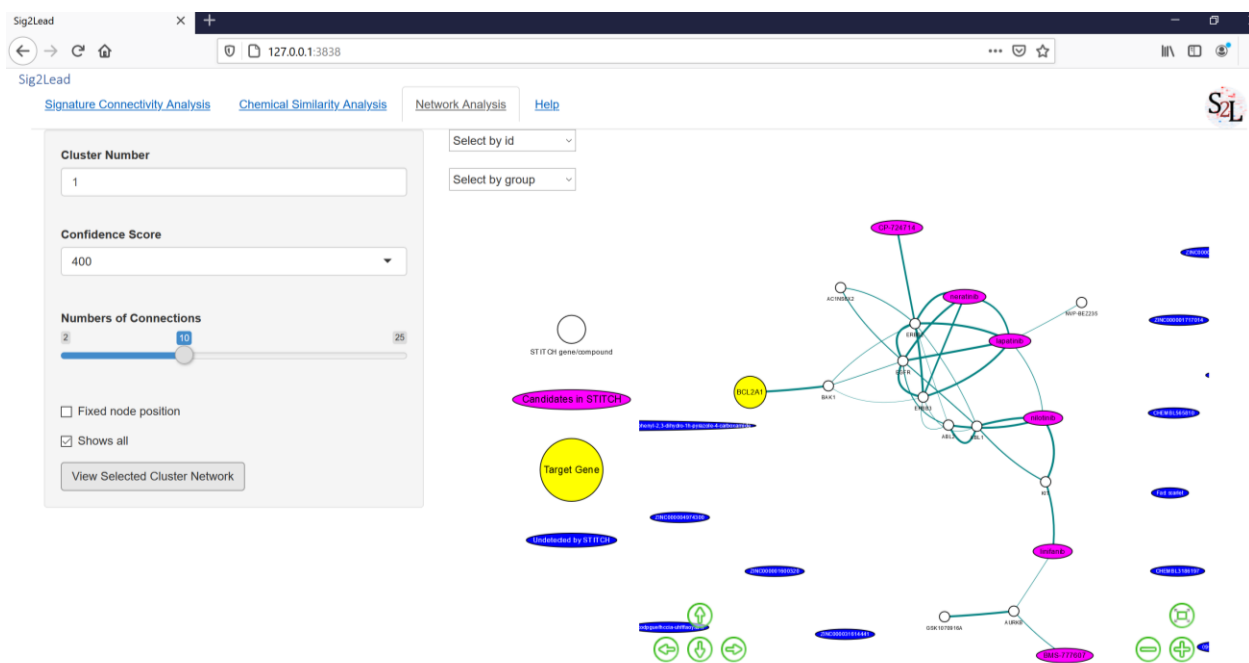


Figure 8: Cluster Network STITCH. Under the STITCH Network analysis, known interactions will be mapped between the target gene and putative compounds submitted via Sig2Lead. The target gene is shown in yellow, other STITCH derived genes within the pathway are shown in white, and compounds identified via Sig2Lead are shown in magenta. The figure shows additional compounds to be considered as putative pathway inhibitors (blue) based on their chemical similarity to known pathway or target inhibitors (magenta).